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Fractionation and Characterization of Dent Corn and Amylomaize Starch Granules*

By J. E. Cluskey, C. A. Knutson and G. E. Inglett, Peoria

Starch granules of dent corn and Classes V and VII amylomaizes were fractionated according to size by an aqueous differential sedimentation procedure. The sized fractions were characterized by microscopy and by a modified method for determining their iodine binding capacities. Results show an inverse relationship between granule size and iodine binding capacity in the amylomaizes. Ordinary dent corn does not show this. The percent apparent amylose in the fractions of amylomaize V starch amounted to 40% for the largest size particles and 52% for the smallest ones; for amylomaize VII starch, 46% for the large particles and 70% for the small particles. Dent corn averaged 23% apparent amylose.

Fraktionierung und Charakterisierung der Stärkekörner aus Zahnmais und Amylomais. Die Stärkekörner von Zahnmais und den Amylomaisklassen V und VII wurden mit Hilfe einer wäßrigen Differential-Sedimentationsmethode fraktioniert. Die getrennten Fraktionen wurden durch Mikroskopie und durch eine modifizierte Methode zur Bestimmung ihres Jodbindungsvermögens charakterisiert. Bei Amylomais zeigen die Ergebnisse eine umgekehrte Beziehung zwischen Korngröße und Jodbindungsvermögen. Gewöhnlicher Zahnmais zeigt dies nicht. Der prozentuale Gehalt an scheinbarer Amylose in der Stärke der Fraktionen von Amylomais V betrug 40% bei den größten und 52% bei den kleinsten Körnern. Bei Amylomais VII waren es 46 und 70%. Zahnmais wies dagegen einen durchschnittlichen Gehalt an scheinbarer Amylose von 23% auf.

1 Introduction

The importance of starch in nutrition as an energy source has continually promoted renewed research effort. Consequently, studies were initiated in our Center to determine how composition and properties of corn starches with differing genetic background are related to nutritional availability and efficiency. High-amylose corn starch incorporated into corn muffins has recently been shown by *Wolf* et al. [1] to be less digestible than dent corn starch. Our particular initial interest was the relationship of granule size to inherent corn starch characteristics. Variations of properties according to granule size have been recognized earlier by other investigators with several other cereal grains. The proposal that the fundamental properties of potato starch are determined essentially by the size of the granules has been made by *Geddes* et al. [2]. Also, the properties of small and large granules isolated from mature barley and wheat starch have been reported by *Evers* and coworkers [3]. *Boyer* et al. [4] found granule size to be of importance to the apparent amylose percentage in starch granules isolated from several maize genotypes 36 days post-pollination.

In our starch studies, commercial dent, waxy maize and amylomaize V and VII starches have been fractionated according to granule size by an aqueous differential sedimentation procedure. The size and shape of the large, intermediate and small sized granules were measured. Granule fractions were analyzed for iodine binding capacities as an indication of apparent amylose content.

2 Materials and Methods

The starches were gifts from the American Maize-Products Company, Hammond, Indiana. Commercially, the dent corn starch is known as Amaizo 100; the waxy maize starch, Amaizo Amioca; and the amylomaize starches, Amaizo Amylomaize V and VII. The two amylomaize starches were reported to contain 50 and 70% amylose, respectively.

2.1 Starch granule separation by size

The starch granules were fractionated by a modification of a sedimentation procedure of *Decker* and *Hoeller* [5]. This aqueous serial decantation method was chosen because it would not alter the granules as nonaqueous media might, nor introduce residues that would preclude feeding studies in later work.

Sixty grams of the starch were sprinkled into 600 ml of distilled water being stirred in a 1-liter tall form beaker. When well dispersed, the slurry was mechanically stirred at moderate speed for 5 min. The stirrer was then removed and the suspension was let rest for 1 h. After this time, the supernatant was carefully decanted into a second beaker, leaving a layer of heavier particles in the original vessel. The first beaker was then restored to the original volume. The contents of the two beakers were stirred for 5 min as originally done and let stand for 1 h sedimentation time. The procedure was repeated for as many transfers as needed to effect good separation. A new beaker was added to the chain at each transfer. When the heavy starch granules sedimented completely after the hour, leaving a water-clear supernatant, the beaker was eliminated from the chain. The supernatant was then decanted and the starch granules were dried over $CaCl_2$ in a desiccator. Such fractions were referred to as residue fractions. The small particles that are carried along in the supernatant were eventually collected at the end of the sedimenting chain, when no obvious residue layer was found after 1 h standing. These small particle slurries were pooled until 3–4 l were collected and were designated composite suspension fractions or fine fractions. These supernatants were next centrifuged in a Sharples centrifuge to reduce volume. The granules remaining in the centrifuge bowl were collected and freeze dried.

Exposure of the starch granules to distilled water for relatively long periods of time had no adverse effect on them. Leaching of carbohydrate into the water did not occur. Phenol-sulfuric acid carbohydrate analysis (*Dubois* et al [6]) of the supernatant after sedimentation showed less than 0.5% carbohydrate.

2.2 Granule size measurements

Granule size measurements were made from photomicrographs (580 × magnification) of the parent starch, of

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selected sedimented granule fractions and of the composite suspended granule fractions. The dimension of these particles were measured visually from the photomicrographs using a 7 power comparator fitted with a calibrated scale. Three classifications, spherical, elliptical and filamentous, were used to designate granule shape. Spherical granules were defined as those with a length-to-width ratio less than 1.3; ellipsoidal granules with a ratio of 1.3 to 3.0; and filamentous, with a ratio greater than 3.0. Average dimensions were calculated from 200 observations from each plate.

2.3 Iodine binding capacity measurements

Materials and equipment for the titrimetric study of the iodine binding capacity of the starches and the fractions were essentially the same as described by *Banks* et al. [7].

The reagents were prepared as follows: The electrolyte solution consisted of a mixture of 203 ml 0.1-M KI and 20 ml 0.2-M KH_2PO_4 + K_2HPO_4 buffer (pH 5.8) diluted with water to 1 liter. The standard iodine solution was 0.025-M iodine in 0.1-M KI. This stock solution was diluted 1 to 10 with water immediately prior to use.

The starch sample was defatted by dissolving 1 g in 100 ml of dimethyl sulfoxide, precipitating with 300 ml absolute ethanol, washing three times with ethanol and drying under vacuum over P_2O_5 overnight at room temperature, then for 1 h at 65 °C.

The defatted starch, 100 mg, was dissolved in 10 ml of 90% DMSO, and 0.8-ml aliquots were diluted to 10 ml with water to provide the titration samples.

The complete titration cell consists of two 1-liter round-bottomed, 3-necked flasks connected by a liquid bridge, each fitted with a platinum electrode connected to a microvoltmeter. The flasks were kept at constant temperature in a water bath maintained by thermostated refrigeration units. Stirring was maintained by magnetic stirrers.

The experimental procedure used was a modification of a semimicro, differential, potentiometric iodine titration described by *Banks* et al. [7]. Their method consisted basically of the addition of incremental amounts of iodine to a given weight of carbohydrate and subsequent measurement of the amount of iodine bound. A complete curve of bound iodine versus free iodine was obtained in the method. Our method is a reversed procedure; the iodine concentration was maintained constant as incremental amounts of starch in solution were added.

The modified procedure was carried out as follows. Electrolyte solution (830 ml) was added to each flask. The liquid level of each was adjusted to that of the water bath, and the liquid bridge was put in place. One milliliter of 0.0025-M iodine reagent was placed in the reaction flask, and enough of the same reagent was added to the reference flask to null the voltmeter (1 ml ± 1–2%). Starch solution aliquots of 0.5 or 1.0 ml were then added to the reaction flask and allowed to equilibrate for 5 min. Any iodine bound by the starch was then replenished, using a micrometer syringe to bring the voltmeter back to the null point. The amount of iodine required, which is equal to the amount bound, was recorded and another aliquot was added. A minimum of 8 aliquots was added for each sample to ascertain linearity of the reaction. After each addition, a sample was removed and the absorbance spectrum was measured from 650–450 nm in a Cary 14 recording spectrophotometer. The sample was then returned to the reaction flask prior to addition of the next aliquot. Experiments were performed at 2 and 20 °C.

Iodine binding capacity (IBC) was calculated from the slope of the curve of a plot of mg iodine bound versus mg starch added. The slope of the curve was determined by a least squares regression analysis. Apparent amylose content was determined by comparison of the IBC to that of pure amylose.

3 Results and Discussion

The dimensions of starch granules from the original starch samples and from some of the fractions are shown in Table 1. Ordinary dent corn starch granules approach, in general, a spherical shape. Approximately three-fourths of the dent corn granules were classified into the spherical-shaped group and one-fourth into the ellipsoidal category. No filamentous granules were observed. Fraction A represents the largest granules isolated in the separation and Fraction H the

Table 1.
Starch Granule Characteristics.

Starch from	Configuration; size (μ); amount (%)									Apparent amylose content[1] (%), 2 °C	
	Spherical			Ellipsoidal			Filamentous				
	l	w.	%	l	w.	%	l	w.	%	By titration	By absorbance
Dent corn											
Unfractionated	11.5	10.3	76	13.2	9.8	24			0	24.6	27.8
Fraction (A)	15.8	14.4	80	16.5	12.6	20			0	24.2	26.9
Fraction (H)	6.1	5.5	74	7.8	5.3	26			0	22.2	26.0
Amylomaize V											
Unfractionated	10.0	8.3	64	11.8	8.5	35	25.9	6.9	1	44.5	54.4
Fraction (A)	15.3	13.4	60	17.3	12.8	40			0	40.5	40.9
Fraction (B)	14.0	12.4	62	16.2	11.7	36	26.6	6.9	2	45.9	52.4
Fraction (Q_1)	6.8	6.2	64	8.0	5.2	35	16.5	31.0	2	48.1	54.3
Fraction (Q_3)	6.5	5.8	62	6.9	5.3	37	12.8	4.1	1	52.0	57.9
Amylomaize VII											
Unfractionated	7.8	7.2	56	9.9	5.8	35	23.2	4.1	9	63.8	70.2
Fraction (A)	12.0	11.2	71	13.7	9.1	26	16.2	4.2	3	45.6	51.6
Fraction (E)	10.5	10.0	69	12.3	7.9	27	15.4	4.0	4	56.6	62.2
Fraction (Q)	6.2	5.7	47	8.9	4.9	42	17.4	2.8	11	62.1	66.3
Fraction (O)	5.0	4.6	52	7.1	4.0	40	16.2	2.1	8	69.6	72.9

1) The standard error of estimate was calculated to be not more than ± 4 mg I_2/100 mg starch.

smallest sizes or fines fraction (Fig. 1). The spherical and ellipsoidal forms of the fines fraction are in about the same ratio as the unfractionated starch, whereas the larger granules have slightly more spherical shapes. The normal dent corn starch, with an average granule size of 11 μ wefore fractionation, yielded fractions varying from 15 to 6 μ.

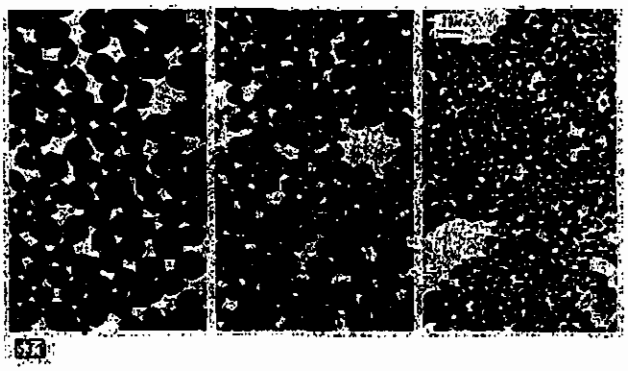

Figure 1. Dent corn starch. Left: Fraction A, the large granules (residue fraction). Center: Unfractionated dent corn granules. Right: Fraction H, the smallest granules (composite suspension fraction).

Figure 2 shows a similar comparison of the fractions from waxy maize starch. Waxy maize starch granules are more angular than those from normal dent corn starch. They were similar in dimension to dent corn starch; however, the smallest granules were larger than the similar dent corn fraction. Waxy maize granules had an unfractionated average size of 12 μ, with fractions varying from 15 to 8 μ.

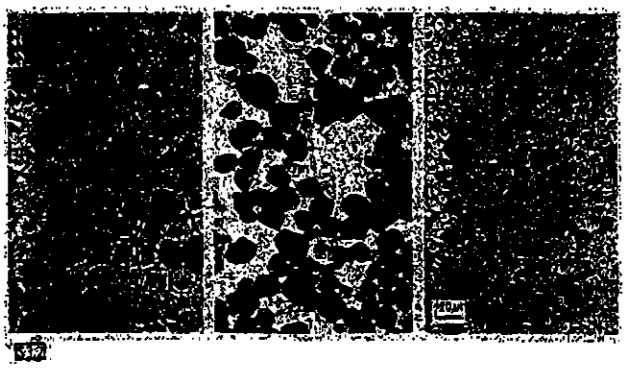

Figure 2. Waxy maize starch. Left: Fraction A, the large granules (residue fraction). Center: Unfractionated waxy maize starch. Right: Fraction H, the smallest granules (composite suspension fraction).

The percentage of spherical granules in the amylomaize starches is not as large as it is for dent corn starch (Table 1). In class V amylomaize, the unfractionated starch as well as the fractions contained 60 – 64% spherical-shaped particles and approximately 30 – 40% elipsoidal-shaped ones. The striking difference is the presence of filamentous starch particles. These unusually shaped granules may be simple or branched filaments of varying length. One or two percent is found in the amylomaize V starch. They are present both in the large and small size fractions; for example, in Fractions B and Q_1 (Table 1).

Figure 3 illustrates the size differences in Amylomaize V starch and the fractions. The particle measurements of the fractions are very similar to those of dent corn fractions. Amylomaize V has an average granule size of 10 μ composed of fractions varying between 15 and 6 μ.

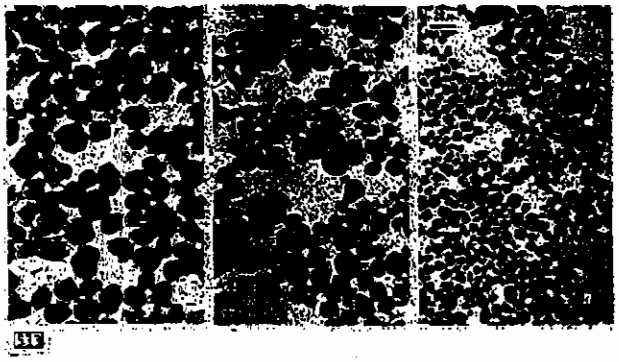

Figure 3. Amylomaize V starch. Left: Fraction A, the large granules (residue fraction). Center: Unfractionated amylomaize V granules. Right: Fraction Q_1, the smallest granules (composite suspension fraction).

In comparison with the other two starches in Table 1, Amylomaize VII starch contains the least number of spherical-shaped granules with the highest percentage of filamentous type. The more readily sedimented fractions, for example, Fractions A and B, contain more of the spherical particles than do the fine fractions, such as Q and O. Conversely, most of the filamentous type granules are retained in the fine fractions. This concentration of the filamentous particles in the fines fraction is shown in Figure 4. On the average, the Amylomaize VII granules measured 8 μ, with fractions varying between 12 and 5 μ.

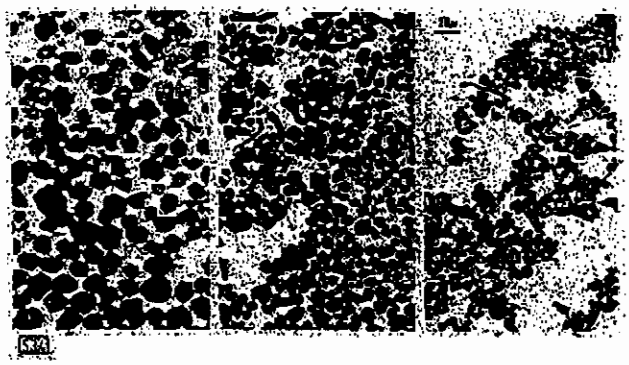

Figure 4. Amylomaize VII starch. Left: Fraction A, the large granules (residue fraction). Center: Unfractionated amylomaize VII granules. Right: Fraction O, the smallest granules (composite suspension fraction).

Average granule sizes of the unfractionated starches were of the same magnitude as those reported by *Wolf* et al. [8] for ordinary dent and amylomaize starch from mature kernels. Although the smallest particle dimension recorded in this work was of the order of 5 μ, the possibility no doubt exists that particles less than 5 μ were present but were lost in the commercial processing of the starch.

3.1 Iodine binding capacity

The iodine binding method of *Banks* et al. [7] was initially used in our investigations. During this time, two problem areas were experienced; namely, (i) the titration data did not remain proportional to the absorbance values at higher concentrations of iodine; and (ii) variation in results from one sample to another of the same material was too great to prove whether there were differences between the fractions, since expected variations were in the range of 5% or less.

described earlier in this paper afforded certain advantages over the original procedure. First of all, a higher degree of reproducibility was established. The amount of iodine bound increased linearly with the amount of starch added (Fig 5). This provided a precise value not dependent upon extrapolation back to a hypothetical value at zero free iodine concentration as required in the original method. The spectrophotometric data collected simultaneously supported well the titration data. Secondly, because of greater binding ability, the modified procedure provided the sensitivity needed to distinguish iodine binding capacity between various size cuts isolated in the sequential aqueous sedimentation. At either of the two temperatures used, 2 and 20 °C, a 1.0 ml volume of iodine was found to be sufficient to provide an excess free iodine concentration such that no change in binding would occur with additional iodine. Accordingly, the values obtained can be described as the maximum iodine binding capacity of the starches. The fact that each starch aliquot was introduced into an excess of free iodine ought to ensure that every binding site is involved.

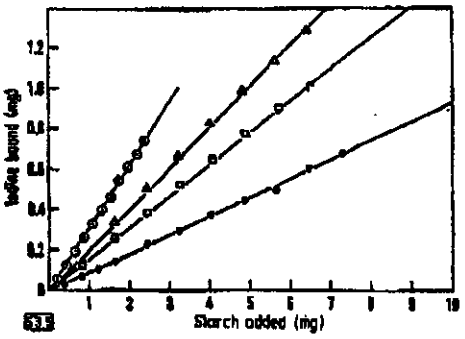

Figure 5. Iodine binding at 2 °C of purified amylose. ⊙; Amaizo 100 (dent), ●, corn starch; and amylomaize V, □; and amylomaize VII, △, starches.

A comparison of data from amylose, dent corn, and amylomaize VII starches derived from both potentiometric methods is given in Table 2. The amount of iodine bound by the amylose and the various starches was considerably higher (ca. 50%) using the modified procedure than when the original procedure was used. The relative amounts of binding capacity appear to remain proportional and, consequently, the resulting apparent amylose values are comparable.

Table 2.
Comparison of Iodine Binding Capacity (IBC) of Starches and Amylose by the Original and Modified Potentiometric Techniques (2 °C).

Sample	Original procedure IBC mg/100	Original procedure Apparent amylose (%)	Modified procedure IBC og/100	Modified procedure Apparent amylose (%)
Amylose	21.8	100.0	31.6	100.0
Dent corn	5.8	26.8	7.6	24.7
Amylomaize VII	14.5	66.5	20.2	63.8

The increased binding that we found in amylose exceeds the 26% bound by amylose in the solid state, which is equivalent to one iodine molecule for each turn of an amylose helix containing six D-glucose units. The standard iodine binding value for amylose in solution, 19.5%, is about 75% of the solid state value, a fact that has never been explained. *Higgenbotham* [9], using a potentiometric method and studying a wide

in solution agreeing with the solid state values. Generally, though, the 19.5% solution value is considered to be a suitable standard under conditions normally used.

It can be argued that steric or charge interference prevents complete filling of the helices when iodine is slowly added to the starch in solution. Perhaps our method, by maintaining a constant amount of excess iodine, forces additional filling of helices by altering the stoichiometry of the bound species. It may be significant that our binding values agree excellently with the amount required to provide one iodine molecule for every five D-glucose residues. Whether such a compact helical conformation is feasible is open to speculation.

Another explanation for the excess binding may be aggregation of amylose-iodine complex molecules. Such aggregation has been suggested by *Foster* and *Paschall* [10] and demonstrated by *Dintzis* et al. [11] by ultracentrifugation. Such intermolecular associations could result in binding more iodine than is found within the helices. If such aggregates are formed, it appears that their composition is quite constant, since we have observed no further binding with increased iodine concentration while maintaining a constant KI concentration. Also, absorbance and titration values remain proportional when iodine concentration is increased. These data will be presented in a later paper; we also intend to investigate the effect of varying the KI concentration.

The average granule dimensions and apparent amylose contents of the parent starches and the largest and smallest particle size distribution fractions also appear in Table 1. This table shows the differences evidenced in the fractionations. Several fractions from the amylomaize separations have been chosen to illustrate the progressive differences found. The dent corn starch shows a normally observed value for amylose, nearly 25%. Absorbance values are found to be somewhat higher. Commercially, the apparent amylose percentage generally is found by using a spectrophotometric method and likely would compare with these values. The (A) fraction contains the largest size granules of the dent corn kernel, approximately 16 μ in diameter, and is very much like the normal dent corn in iodine value. The smallest particle size isolated differs by only 2%. These results are comparable to those reported for mature barley and wheat starches by *Evers* et al. [3]. These investigators found no significant difference between amylose contents of large and small granules.

In Table 1, however, a significant difference may be seen between apparent amylose content of small and large granules of amylomaize starches. With both amylomaize V and VII, the data clearly show an inverse relationship between granule size and iodine affinity. In amylomaize V, Fraction (A) granules (the largest) had an apparent amylose content of 40% while the smallest (Q_3) contained 52%. This inverse relationship is shown in greater extent with the amylomaize VII granules, i. e., 46% for the largest (A) and 70% for the smallest (O). Slightly higher values were obtained throughout by spectrophotometry, but the variation was proportional. As noted by *Adkins* and *Greenwood* [12], we also found that the 20 °C values generally were lower than the 2 °C values.

The results point to the fact that preferential sites of amylose density in amylomaizes are found to exist in the smallest starch particles. As the average granule size decreases in each of the measured shape categories, the apparent amylose value of the amylomaize starch increases. Correlation of particle shapes with amylose value cannot be ascertained by the data, but we do know that filamentous granules are suspected to have a higher proportion of amylose than other

starch bodies as evidenced by their isotropicity under polarized light.
In contrast to the work of *Wolf* et al. [8] who found no obvious correlation between apparent amylose content and average starch granule size in amylomaizes, we propose that a difference does exist. Further characterization of granule fractions and development of our modified iodine binding procedure are in progress.

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The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

Bibliography

[1] *Wolf, M. J., U. Khoo* and *G. E. Inglett:* Stärke **29** (1977), 401.
[2] *Geddes, R., C. T. Greenwood* and *S. Mackenzie:* Carbohydr. Res. **1** (1965), 71.
[3] *Evers, A. D., C. T. Greenwood, D. D. Muir* and *C. Venables:* Stärke **26** (1973), 42.
[4] *Boyer, C. D., J. C. Shannon, D. L. Garwood* and *R. G. Creech:* Cereal Chem. **53** (1976), 327.
[5] *Decker, P.,* and *H. Hoeller:* J. Chromatogr. **7** (1962), 392.
[6] *Dubois, M., K. H. Gilles, J. K. Hamilton, P. H. Rebers* and *F. Smith:* Anal. Chem. **28** (1956), 350.
[7] *Banks, W., C. T. Greenwood* and *D. D. Muir:* Stärke **23** (1971), 118.
[8] *Wolf, M. J., H. L. Seckinger* and *R. J. Dimler:* Stärke **16** (1964), 375.
[9] *Higgenbotham, R. S.:* J. Text. Inst. Trans. **40** (1949), T-783.
[10] *Foster, J. F.,* and *E. F. Paschall:* J. Am. Chem. Soc. **74** (1952), 2105.
[11] *Dintzis, F. R., A. C. Beckwith, G. E. Babcock* and *R. Tobin:* Macromolecules **9** (1976), 471.
[12] *Adkins, G. K.,* and *C. T. Greenwood:* Carbohydr. Res. **3** (1966), 152.

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Studies on Varagu Starch

By S. V. Paramahans, D. B. Wankhede and R. N. Tharanathan, Mysore

Starch has been isolated from the millet varagu in 63% yield. Study of the physical properties revealed: moderate swelling and low solubility power in water; extensive solubility in dimethyl sulfoxide, probably indicative of easy solvent penetration due to labile and heterogeneous bonding forces within the granule. Brabender amylogram indicated little retrogradation on cooling, owing to strong bonding between the linear and branched molecules of starch. The amylose content of the starch was 24% as determined by paper chromatographic fractionation and "Blue value" method.

Untersuchungen über Varagustärke. Aus Varagu-Hirse wurde Stärke in 63%iger Ausbeute isoliert. Die Untersuchung der physikalischen Eigenschaften ergab: mäßige Quellung und niedriges Lösungsvermögen in Wasser, hohe Löslichkeit in Dimethylsulfoxid, die möglicherweise auf leichte Durchdringbarkeit mit Lösungsmitteln auf Grund labiler und heterogener Bindungskräfte innerhalb des Korns hinweist. Das Brabender-Viskogramm zeigte geringe Retrogradation bei der Abkühlung infolge starker Bindung zwischen den linearen und verzweigten Molekülen der Stärke. Der Amylosegehalt der Stärke betrug 24%, wie durch papierchromatographische Fraktionierung und die „Blauwert"-Methode festgestellt werden konnte.

1 Introduction

There are few reports in the scientific literature on the nature and composition of the starchy and non-starchy polysaccharides of millets [1–7]. Millets are nutritionally potential grains consumed by a large section of people of rural parts. Variety of millets are grown and consumed in several parts of India. Varagu (*Paspalum scrobiculatum*) is one such millet used in Tamil Nadu state.
Recently we have investigated the carbohydrate composition of some of the millets like ragi (finger millet, *Eleusine coracana*), navane (foxtail millet, *Setaria italica*) [2], varagu (*Paspalum scrobiculatum*) [8], and samai (*Panicum milaceum*) [9]. From the results is apparent that not much variation exists in the carbohydrate profile of these and other millets. However, a detailed study of millet starches is interesting and useful from the chemical and technological point of view. The present investigation deals with the isolation and properties of starch from the grains of varagu, which constitutes the major bulk of the millet.

2 Materials

2.1 Varagu Grains

The millet grains were obtained from the University of Agricultural Sciences, Coimbatore, Tamil Nadu. The grains were dehusked in a centrifugal sheller and the husk portion separated in a laboratory McGill aspirator [8].

2.2 Isolation and Purification of Starch

Crude starch was isolated by steeping the grains in water (100 g/0.5 l, 12 h, 5 °C) and homogenizing in a waring blender (in the presence of 0.01-M mercuric chloride solution). Sieving the homogenate yielded crude starch containing over 2% protein. Purification was done by repeated shaking of a suspension of the starch in 0.2-M NaCl – toluene (1:0.2, by vol.). The purified preparation had the chemical characteristics given in Table 1.